Analytical Methods

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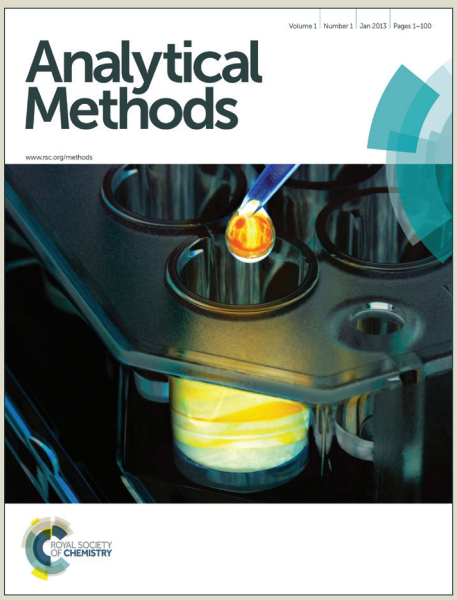

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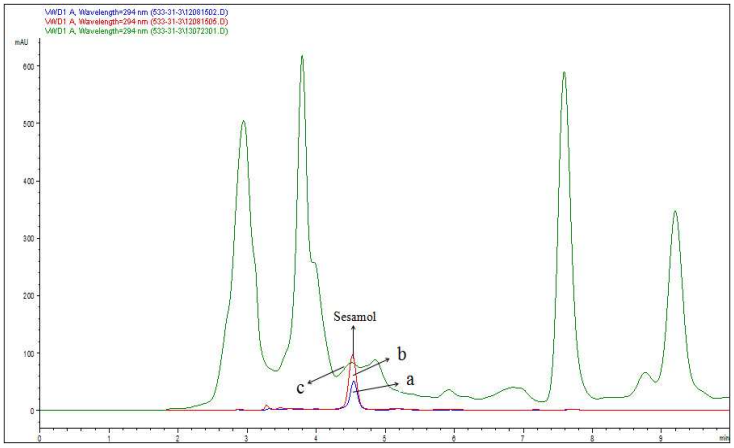
Highlight

Novelty sensitive and reliable anion exchange SPE procedure method for cleanup and extraction of sesamol in sesame oil was developed.

Colour graphic



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ARTICLE TYPE

Determination of Sesamol in Sesame Oil by Anion Exchange Solid Phase Extraction Couple with HPLC

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An anion exchange solid phase extraction (SPE) procedure coupled with high-performance liquid chromatography was developed for the determination of sesamol in sesame oil. Sesamol was effectively extracted from sesame oil using a Cleanert PAX (500mg/6ml) anion exchange SPE column. Conditions of SPE procedure including type, volume, and pH of sample loading solvent, volume and pH of washing solution, and type, volume, and pH of eluent were optimized for efficient sample cleanup with excellent sesamol recovery. The calibration curve of sesamol showed excellent linearity in the range of 10-500 $mg \cdot l^{-1}$ with correlation coefficient (r^2) of 0.9996. The SPE-HPLC method demonstrated high reproducibility with precision values (relative standard deviations, RSDs) of 1.9%-8.2% and high recoveries in the range of 88.2%-106.1% for spiked sesame oil samples. The limit of quantitation (S/N=10) was found to be 5.0 $mg \cdot Kg^{-1}$. The optimized method was successfully applied to the determination of sesammol in sesame oil samples from a local market.

1. Introduction

Sesame (Sesamum indicum) seed oil is high-priced, high-quality edible oil with a unique flavor. It is widely consumed in India and China as a traditional health food.[1] Despite its high degree of unsaturation, sesame oil is one of the most stable edible oils. It is rich in natural antioxidants in from of lignans, which account for this superior stability.[2] Compounds in sesame seed oil that are responsible for its health benefits and antioxidant properties have attracted considerable interest from research scientists.[3]

Sesamol (3,4-methylenedioxyphenol), a main form of lignans in sesame oil, is of particular significance for its antioxidant activity.[4] Sesamol has been reported to act as a metabolic regulator and exhibit anti-aging, antitumor, antimutagenic, anticarcinogenic, and hepatoprotection properties.[5] Sesame oil is mainly produced and consumed in Asian countries such as China and India. Sesame oil production in China and India accounts for nearly half of its total production in the world. In China, sesame oil with a dark color and strong flavor is especially valued. Sesamol content plays an important role in the color and flavor of sesame oil,[6] therefore determination of sesamol in sesame oil is important in evaluation of sesame oil.

Sesamol is a polar compound with low molecular weight and high volatility and is usually determined by reverse phase high-performance liquid chromatography (RP-HPLC) coupled diode-array detection[7,8] or ultraviolet (UV) detection.[9-12] However, due to the complexity of sesame oil matrices, cleanup and enrichment procedures are often required prior to sesamol analysis. Sesame oil contains high contents of triglycerides (TGs), pigments, free fatty acids (FFAs), and peroxides.[13] When crude samples are directly injected into RP-HPLC without cleanup, large amounts of matrix substances are strongly adsorbed by nonpolar octadecyl gels in the RP-HPLC column, resulting in poor resolution and inaccurate quantification of sesamol.[9-12] Thin-layer chromatography (TLC) has been used to cleanup sesame oil samples for sesamol analysis.[3,9,14] However, recovery of sesamol from TLC separation is poor owing to high volatility of sesamol. Solid-phase extraction (SPE)[15-17] has advantages of high recovery, low solvent consumption, and quick and easy operation and therefore is widely used for sample cleanup. The type of adsorbent used in SPE procedure is a critical factor affecting efficiency in cleanup and enrichment. Few materials have been used as SPE adsorbents for cleanup of sesame oil samples for sesamol analysis. Huang et al. [18] reported using neutral alumina as an SPE sorbent for cleanup of sesame oil samples; however, recovery of sesamol was not satisfactory. An SPE adsorbent that effectively separates sesamol from matrix substance in sesame oil is needed for quick and accurate sesamol determination. SPE columns packed with anion exchange adsorbents bound with quaternary ammonium salts have been used to extract and enrich phenols in water samples[19-21] and endocrine-disrupting phenols in sewage[22]. Since sesamol is a derivatized phenol, we thought that anion exchange SPE might also be suitable for extraction and enrichment of sesamol in sesame oil. To date, no reports on the use of anion exchange SPE for cleanup of sesame oil samples have been published.

In this study, we report for the first time an SPE procedure for sesame oil cleanup using a stationary phase of anion exchange adsorbents bound with quaternary ammonium salts. Sesame oil samples were cleaned up using the SPE procedure and subjected to HPLC-UV analysis for determination of sesamol content. Several key factors affecting SPE performance including the type, volume, and pH of sample loading solvent, volume and pH of washing solution, and type, volume, and pH of eluent were optimized to achieve efficient cleanup with high recovery. The method was successfully used to determine sesamol content in sesame oil samples.

2. Experimental

2.1. Chemical reagents and materials

Sesamol standard (25 g, 99.0%) was purchased from Tokyo Chemical Industry (TCI, Tokyo, Japan). A stock solution of 5 $g \cdot l^{-1}$ sesamol was prepared in methanol. Standard solutions were prepared by dilution of the stock solution with methanol prior to analysis. All solvents, including methanol (MeOH), ethanol, acetonitrile, ethyl acetate, *n*-hexane and acetic acid (AA), were of HPLC grade and purchased from Tianjin Kermel Chemical Co. (Tianjin, China). Potassium hydroxide (KOH) and potassium phosphate monobasic (KH_2PO_4) were of G.R. grade and also from Tianjin Kermel Chemical Co.., a 0.5 M KOH-ethanol solution was prepared. Water was purified on a Synergy 185 ultrapure water system (Millipore, USA). Cleanert PAX anion exchange SPE extraction columns (500mg/6mL) were purchased from Bonna-Agela Technologies (Tianjin, China). pH of solutions was determined on a pH meter with precision of 0.1 (PHS-3C, INESA Instrument Co., Shanghai, China). Sesame oil samples were purchased from a local market.

2.2. Solid-phase extraction procedures

Sample loading solution was prepared by dissolving 0.5 g sesame oil in 20 ml ethanol. pH of the solution was adjusted to 12.0 using 0.5 M KOH-ethanol solution. An SPE cartridge was connected to Visiprep^{TM} SPE vacuum manifolds (Supelco, USA). The cartridge was preconditioned with 10 ml of 0.5 M KOH-ethanol solution (pH 12) to remove air and leach impurities. Sesame oil sample solution prepared as 20 ml was then loaded to the preconditioned cartridge. Following a 5ml ethanol wash, sesamol retained on the cartridge was eluted with 5.0 ml AA-methanol solution (pH 3.0). The eluate was collected into a test tube and condensed to 1.0 ml under a gentle flow of nitrogen at room temperature. The solution was then transferred to double layer silicone-teflon septa vials, placed in an autosampler, and analyzed by HPLC.

2.3. HPLC analysis

HPLC analysis were performed on an HPLC system (1100, Agilent Co., USA) equipped with a UV detector. A Zorbax SB-C18 column (250mm×4.6mm×5μm, Agilent Co.) thermostated at 35 ℃ was used for sesamol analysis. Samples (10 μl) were injected and eluted with an isocratic mobile phase of 60% methanol and 40% KH_2PO_4-water solution (0.05 M) at flow rate of 0.8 mL/min. Sesamol was detected by absorbance at 294 nm.

Calibration curve was obtained using five standard solution of sesamol (10.0, 50.0, 100.0, 200.0, and 500.0 $mg \cdot l^{-1}$) prepared by serial dilution of a stock solution (5 $g \cdot l^{-1}$). Sesamol was quantified according to the calibration curve.

3. Results and discussion

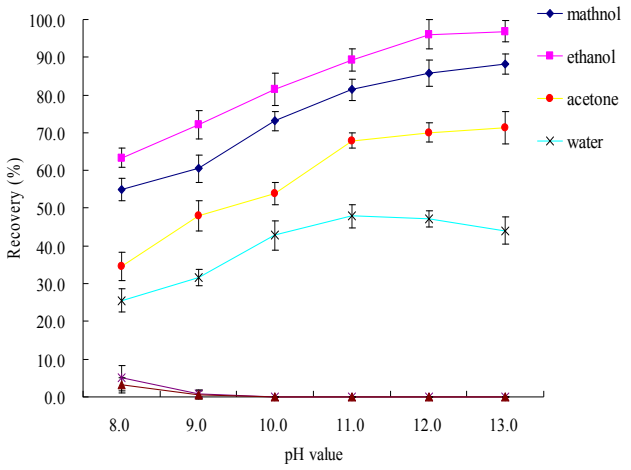
3.1. Results

Recovery of analyte from SPE is affected by several factors such as the solvent for sample loading, the pH and volume of washing solution, and the pH and volume of eluent. In this study, these factors were investigated using a spiked refined soybean oil sample (100 $mg \cdot Kg^{-1}$) and optimized for high analyte recovery. All experiments were conducted in triplicates.

3.1.1. Study of sample solvent

Extraction efficiency of the anion exchange SPE procedure depends on the solvent used to dissolve sesame oil samples. In six difference pH conditions (8.0, 9.0, 10.0, 11.0, 12.0, and 13.0), we tested six solvents with different polarity including methanol, ethanol, acetone, ethyl acetate, n-hexane, and water as solvents for sample dissolution. Recoveries of sesamol obtained are shown in Fig. 1. Our results showed that the highest recovery was obtained with ethanol as the dissolution solvent (96.8%) followed by methanol (88.2%), acetone (71.3%), and water (44.0%). Recoveries obtained with ethyl acetate and n-hexane were less than 5.0%. We also tested the effect of different volumes of dissolution solvent on recovery. Recoveries obtained with 10, 20, 30, and 40 ml of ethanol as sample dissolution solvent were 87.2%, 97.8%, 96.5% and 95.7%, respectively.

Fig. 1



Effects of sample loading solvent and pH on sesamol recovery. SPE was performed under sample loading volume of 20ml, 3.0 ml ethanol as washing solvent, and 7 ml methanol (pH 3.0) as eluent.

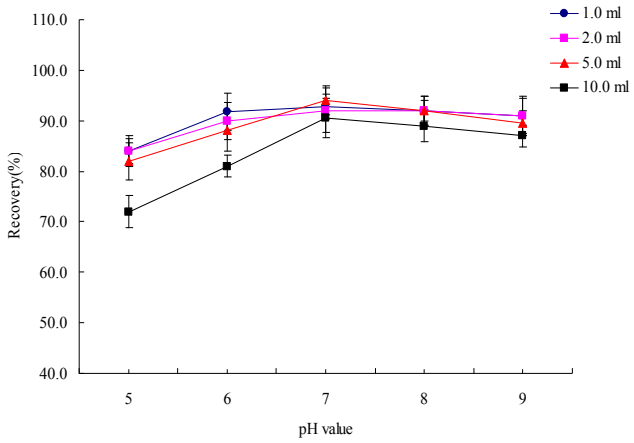
3.1.2. Study of washing solvent

Extraction efficiency of the SPE procedure also depends on volume and pH of washing solvent. We assessed sesamol recovery using 1, 2, 5, and 10 ml ethanol at pH 5.0, 6.0, 7.0, 8.0, and 9.0 as washing solvent (Fig. 2).The highest recovery was obtained using washing solvent of 5 ml ethanol at pH 7.0 (97.2%).

3.1.3. Study of eluent

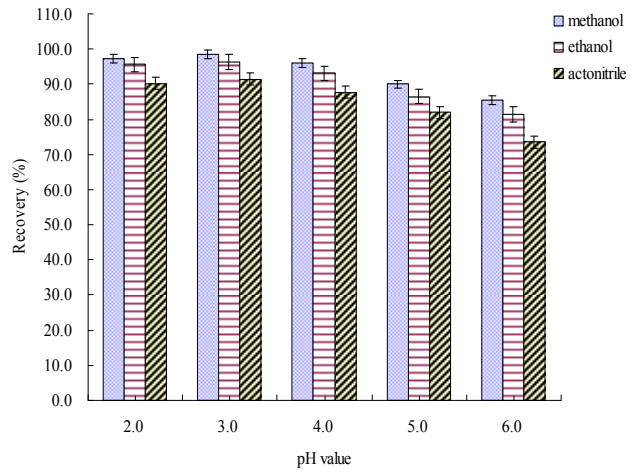
Since sesamol is weakly acidic, pH of eluent greatly affects its recovery. We tested methanol, ethanol, and acetonitrile as eluent under different pH conditions (Fig. 3). Our results indicated that highest recoveries were obtained with methanol at pH 2.0 and 3.0 (97.1%-98.5%). We also studied sesamol recovery using different volumes of methanol at different pH conditions as eluent (Fig. 4) and found that the highest recovery was achieved with 5.0 ml of methanol at pH 2.0.

Fig. 2



Effects of volume and pH of washing solvent on sesamol recovery. SPE was performed using ethanol as loading solvent with sample loading volume of 20 ml at pH 12.0. Other conditions were identical to those described in Fig. 1.

Fig. 3



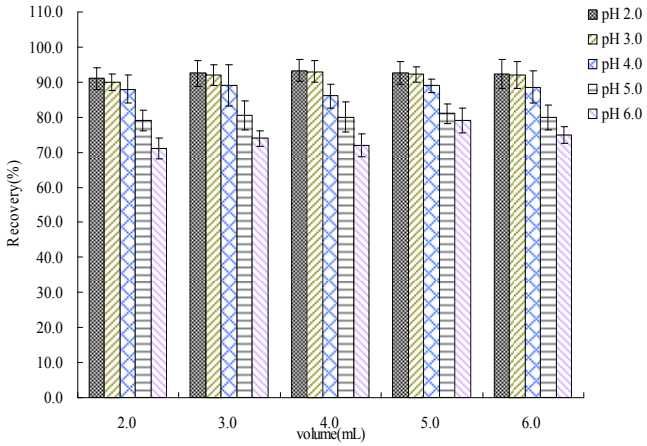
Effects of eluent and pH on sesamol recovery. SPE was performed using 5 ml ethanol (pH 7.0) as washing solvent. Other conditions were identical to those described in Fig. 1.

3.2. Discussion

3.2.1. Effect of sample solvent

Among the six solvents tested for sample dissolution, sesame oil was completely dissolved in ethanol, acetone, ethyl acetate, and n-hexane. However, high recovery was only obtained with ethanol (96.8%). Recovery with acetone was lower than 71.3%, probably because sesamol was poorly dissociated in acetone. Similarly the lower than 5% recovery with ethyl acetate and n-hexane was likely attributed to its low polarity, which caused poor dissociation of sesamol. Since sesame oil is not soluble in methanol or water, when methanol or water was tested as loading solvent, sesame oil was extracted with methanol or water. Methanol or water layer was collected after centrifugation and loaded to SPE cartridge. Recovery obtained with methanol (88.2%) was lower than that with ethanol (96.8%) probably because some sesamol was lost during the extraction process. The low recovery obtained with water (44.0%) was also likely caused by poor extraction because water did not separate well from oil owing to emulsification. Fig.1 shows that the highest recovery was obtained when pH of the sesame oil sample solution in ethanol was adjusted to 12.0. It suggested that sesamol was fully dissociated under this strong alkaline condition. Further tests showed that sample dissolved in 20 ml ethanol yielded the highest recovery. Therefore we selected to use 20 ml ethanol as loading solvent and adjust pH of the oil-ethanol loading solution to 12.0 for sample loading.

Fig. 4



Effects of pH and volume of methanol as eluent on sesamol recovery. SPE was performed using 5 ml ethanol (pH 7.0) as washing solvent. Other conditions were identical to those described in Fig. 1.

3.2.2. Effect of washing solvent

Dissociated sesamol may change back to neutral form in acidic washing solvent and be washed off during the washing step. Indeed, low recovery was obtained with acidic washing solvent, especially when pH of washing solvent was lower than 5 (Fig. 2). Recovery was also low in alkaline washing solvent because alkaline solvent was unable to remove acidic matrix substances such as FFAs, which may interfere with subsequent HPLC analysis. Thus the highest recovery (97.2%) was obtained using 5.0 ml ethanol at neutral pH (pH 7.0) as washing solvent.

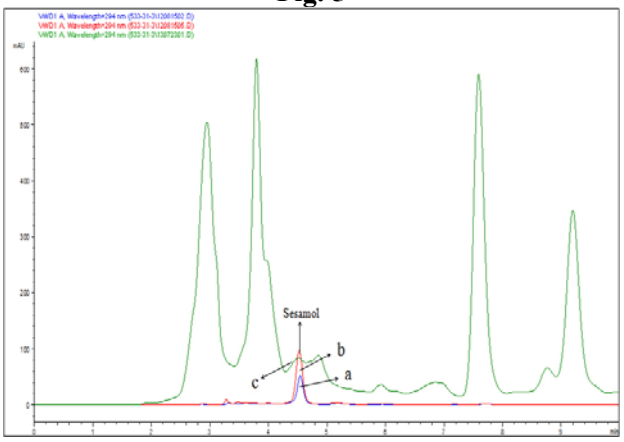
3.2.3. Effect of elute

As Fig. 3 shows, highest recoveries (97.1%-98.5%) were obtained using methanol as eluent at pH 2.0 or 3.0, probably because methanol exhibits higher polarity than ethanol and acetonitrile. Considering that commercially available C18 HPLC columns are usually run within pH range of 2–8 to ensure good performance within their life span, we selected to use 5.0 ml methanol at pH 3.0 (AA-methanol solution) as eluent for SPE procedure.

3.3. Performance and application of SPE-HPLC method

Performance of the SPE-HPLC method for sesamol determination was evaluated under optimized SPE conditions including 20 ml ethanol as dissolution solvent and an oil-ethanol solution at pH 12.0 for sample loading, 5.0 ml ethanol at pH 7.0 as washing solvent, and 5.0 ml methanol at pH 3.0 (AA-methanol solution) as eluent. The calibration curve of sesamol obtained by least-squares linear regression analysis of peak area versus concentration of standard solution showed excellent linearity in the range of 10-500 $mg \cdot l^{-1}$ with correlation coefficient (r^2) of 0.9996. The limit of quantitation (LOQ) was calculated to be 5.0 $mg \cdot Kg^{-1}$ or 2.5 $mg \cdot l^{-1}$ based on a signal-to-noise ratio of 10. Precision (RSD) and recovery were assessed by spiking three concentrations of sesamol (20.0, 200.0, and 400.0 $mg \cdot Kg^{-1}$) in sesame oil purchased from a local market, those three samples were a certain brand of sesame oil which was produced by different varieties of sesame seeds from different countries. Average recoveries and RSDs were calculated over five repeated tests at each spiking level (Table I). Recoveries of sesamol ranged from 88.2% to 106.1% with RSDs of 1.9% to 8.2%. Then we tested sesame oil samples from the local market using the optimized SPE-HPLC method. Chromatograms of sesamol oil sample (a), sesame oil sample (b) spiked with 200.0 $mg \cdot Kg^{-1}$ sesamol and sesame oil sample (c) without SPE treatment are shown in Fig.5. As shown in Fig. 5, sesamol was not separated or detected by HPLC if crude sesame oil sample was subjected to HPLC analysis without SPE cleanup. As shown in Table I, sesamol content in market sold sesame oils was determined to be 197.8 $mg \cdot Kg^{-1}$, 267.5 $mg \cdot Kg^{-1}$ and 307.8 $mg \cdot Kg^{-1}$ respectively. Therefore, the anion exchange SPE coupled HPLC method for sesamol determination was sensitive, reproducible, and easy to perform.

Fig. 5



HPLC chromatograms of sesamol in (a) market sold sesame oil sample and (b) spiked sesame oil sample (200 $mg \cdot Kg^{-1}$), and (c) crude sesame oil sample without SPE treatment. SPE was performed using 20 ml oil-ethanol solution at pH 12.0 as sample loading solution, 5 ml ethanol (pH 7.0) as washing solvent, and 5 ml methanol (pH 3.0) as eluent.

Conclusions

An anion exchange SPE procedure was developed to extract sesamol from sesame oil samples for HPLC quantification of sesamol. The SPE procedure efficiently extracted sesamol and removed matrix substances interfering with HPLC analysis. The anion exchange SPE coupled HPLC method enabled selective and sensitive analysis of sesamol in a complex oil matrix environment. The method was demonstrated to be suitable for routine analysis of sesamol content in sesame oil.

Table I

Sample	Real Concentration ($mg \cdot Kg^{-1}$) [a]	Add ($mg \cdot Kg^{-1}$)	Found[b] ($mg \cdot Kg^{-1}$)	Recovery[c] (%)	RSD[d] (%)
		20.0	219.8	106.1	5.9
1	197.8	200.0	396.1	99.5	1.9
		400.0	592.9	98.8	2.0
		20.0	273.4	95.1	8.2
2	267.5	200.0	478.2	102.3	3.5
		400.0	715.6	101.1	2.7
		20.0	289.3	88.2	7.9
3	307.8	200.0	521.6	102.7	4.6
		400.0	688.5	97.3	5.1

Method precisions and recoveries of sesamol.

[a] Seamol concentration in real sesame oil samples, $n = 15$.

[b] Average detected of five replicate runs.

[c] Average recovery of five replicate runs.

[d] Average RSD of real spiked sample recoveries, $n = 5$.

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Notes and references

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